Evaluating the presence of human pathogens in commercially frozen, biologically appropriate raw pet food sold in Italy

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Abstract

Background Biologically appropriate raw food (BARF) diet is becoming more and more popular among pet owners in Europe. However, there are documented microbiological risks associated with raw feeding, and this study aimed to determine the presence of human pathogens in commercially frozen BARF products sold in Italy.

Methods *Salmonella* species, *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Campylobacter* species were identified. The general microbiological quality of BARF products and hygiene were also evaluated. Sample size was limited and therefore the study may not be representative of a larger sample.

Results None of the tested samples showed total bacterial count (TBC) higher than the limit set to consider a sample unacceptable. However, 14 out of 21 samples showed TBC higher than the limit set to consider a sample marginally acceptable. A high percentage of samples were contaminated by the aforementioned pathogens, highlighting the need for pet owners to be aware of the risks of this feeding strategy both to themselves and to their pets.

Conclusions Considering that BARF diet meals can be prepared at home using the hands, as well as tools and spaces that could be shared, guidelines on safer handling of these pet food products should be recommended by veterinarians and nutritionists.

Introduction

Biologically appropriate raw food (BARF) diet is becoming more and more popular among pet owners.[1] This type of diet recently gained popularity as a means to provide energy and nutrients to companion animals. It is based on products such as raw meat, organs and bones, fish, as well as unpasteurised milk and raw eggs, and can be administered as such or after grinding. Commercial BARF diets are generally supplied as frozen products and are available online.[2] Several benefits have been proposed for pets fed with BARF diets[2–5]; however, majority of these remain anecdotal and not supported by highly relevant data.[6] In addition to lack of studies that strongly prove their nutritional benefits,[7] given the frequency with which raw animal products are contaminated with foodborne pathogens, feeding BARF to pets has been cited as a potential risk factor to human and animal health.[8–11] People can be exposed to pet-associated risk factors directly by petting the animals or indirectly through pet food or handling contaminated objects.[12–14] So far, the focus has been mainly on the presence of zoonotic bacteria[15–17] and antibiotic-resistant bacteria. Van Bree *et al*[18] studied the presence of parasites in BARF diets. Of 35 samples, they detected *Sarcocystis* species and *Toxoplasma gondii* DNA in eight and two samples, respectively. Nevertheless, as they have concluded, such a finding in frozen products does not represent a risk neither to people nor to pets since the parasites are inactivated by freezing.

Most studies on bacterial contamination in BARF diets have been conducted in Canada and the USA, while limited information is available regarding products in European countries,[11 18 19] where the recovery of pathogenic bacteria has been the cause of several withdrawals of raw pet food.

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Only recently, a study on the prevalence of *Escherichia coli* and *Salmonella* and the frequency of occurrence of extended-spectrum beta-lactamase (ESBL) producing isolates in Italian raw meat products for pets have been published.[20]

The number of pets in Italy is estimated at 60,400,000, including fish, birds, dogs and cats. About 67 per cent of Italians have at least one pet, positioning the peninsula in the third place in terms of global ranking of 'pet-friendly' European countries.[21] Different from the USA,[22] objective survey data on BARF use in Europe are scarce, but business and expert opinion indicates substantial and growing raw-feeding practices.[23] In Italy, the growth in raw-feeding practices is highlighted by the increase i social media groups dedicated to BARF, counting thousands of participants, and by the constant requests to veterinary nutritionists for diets based on fresh food. There are however documented microbiological risks to animals and people associated with raw feeding,[6] and the aim of this study was to determine the presence of the main pathogenic bacteria contaminants in raw meat, that is, *Salmonella* species, *E coli* O157, *Listeria monocytogenes* and *Campylobacter* species, in commercial BARF products sold in Italy. The general microbiological quality (total bacterial count (TBC) and coliforms) of the BARF products were also evaluated.

Table 1 Declared composition of tested BARF samples

	Brand	Composition
1	A	35% horse meat off-cuts, 25% horse cartilage (sternum), 20% horse offal (lung, heart), 10% horse fat, 10% vegetables (carrots), enriched with salmon oil <1%
2	A	10% omasum, 25% green tripe, 15% beef cartilage, 20% beef cuttings, 25% beef offal (kidney, lung, heart, liver), 5% pureed fruit/vegetables (carrots, apples)
3	A	100% beef
4	A	100% beef green tripe (rumen)
5	A	100% beef muscles
6	A	60% rabbit meat, 40% rabbit carcase
7	A	40% organic beef larynx, 40% green tripe, 20% udder
8	A	100% organic carcases and chicken necks
9	A	75% poultry carcases (chicken, turkey), 25% poultry offal (chicken, turkey), enriched with <1% fish oil
10	B	100% chicken necks
11	B	Horse meat composed of lean cuts of muscle, lung and tripe
12	B	40% beef liver, 40% lung, 20% heart and spleen
13	B	89% lamb and rabbit meat, 8% of bones and cartilage, 3% internal organs
14	B	40% beef meat and heart, 38% fat, 20% trachea, lung and spleen, 2% fresh blood
15	B	100% beef green tripe (rumen)
16	C	100% horse meat
17	C	100% beef tripe (rumen)
18	C	100% beef muscles
19	C	100% chicken back
20	C	100% beef meat and cartilage (epiglottis)
21	C	100% rabbit muscles

BARF, biologically appropriate raw food.

Materials and methods

Twenty-one samples were purchased from three different online stores for BARF products, which are among the most popular stores in Italy. Meat were declared to be butchered in Italy, or bred and slaughtered in Germany, and were produced and commercialised with high-quality standards, in compliance with the EU regulations. Tested products were made of meat and/or by-products of single or multiple animal species, as shown in table 1. Products were shipped frozen directly to the laboratory and stored according to label recommendations until analysis. None of the raw meat products had instructions for thawing or preparation. Before the analysis, samples were thawed at 4°C and processed cold to avoid microbial growth. Each sample was analysed in two replicates. Each analysis was made following hygiene/health and safety regulations and maintaining sterile and asepsis conditions.

Total microbial count and coliforms

Of each sample, 25 g was collected homogeneously under sterile conditions using sterile spoon and transferred into a sterile blender bag (Oxoid, Basingstoke, Hampshire, UK). After the addition of 225 ml of Ringer's solution (Sigma Aldrich, Milan), samples were homogenised using Stomacher (VWR, Milan, Italy) at 350 *g* for 120 seconds. After homogenisation, tenfold serial dilutions of each sample were made in Ringer's solution up to 10^{-7}. Dilutions were then inoculated on to specific culture media. Total microbial count was obtained by plating onto plate count agar (Oxoid) and incubating the plates at 37°C±1°C for 48 hours. Total coliforms were determined by plating the dilutions onto violet red bile agar (Oxoid).

E coli O157:H7

The presence of *E coli* O157:H7 in the samples was evaluated according to ISO 16654-2:2001,[24] with slight modifications. Briefly, 25 g of each sample was aseptically collected and transferred into a sterile blender bag for enrichment with 225 ml of modified tryptone soya broth plus novobiocin (VWR-Merck, Milan). After homogenisation, samples were transferred to a sterile bottle and incubated for 18–24 hours at 41.5°C±1°C. After enrichment, 0.1 ml was spread onto sorbitol MacConkey's agar with cefixime-tellurite supplement (CT-SMAC) (VWR-Merck) and CHROMID O157H7 selective agar (bioMérieux Italia, Firenze). Plates were incubated for 24–26 hours at 37°C±1°C. Typical *E coli* O157:H7 colonies, appearing with green-blue colour on CHROMID O157H7 agar while smooth and colourless with a possible orange halo on CT-SMAC agar, were streaked onto nutrient agar and incubated at 37°C for 18–24 hours. Presumptive *E coli* O157:H7 colonies were confirmed by indole test (VWR Chemicals, Milan) and Microgen *E coli* O157:H7 latex agglutination test (Microgen, UK).

Salmonella species

The presence of *Salmonella* species in the samples was evaluated according to ISO 6579:2002,[25] with slight modifications. Briefly, 25 g of each sample was aseptically collected and transferred to a sterile blender bag for pre-enrichment with 225 ml of buffered peptone water (VWR Chemicals). After homogenisation, samples were transferred to a sterile bottle and incubated for 24±2 hours at 37°C±1°C. Enrichment was carried out by diluting 1 ml from the pre-enrichment bottle in 10 ml of Muller-Kauffmann tetrathionate novobiocin broth (VWR-Merck) and 0.1 ml in 10 ml Rappaport Vassiliadis soya broth (VWR Chemicals). Tubes were incubated, respectively, at 37°C±1°C for 24±3 hours and 41.5°C±1°C for 24±3 hours. Then, 0.1 ml from each enrichment tube was spread onto two selective media: xylose lysine deoxycholate agar (XLD agar) (VWR-Merck) and Rambach agar (VWR-Merck). Plates were incubated for 24±3 hours at 37°C±1°C. Suspected *Salmonella* colonies, appearing with black centre and a reddish zone with a slight transparency on XLD agar and pink on Rambach agar, were seeded onto triple sugar iron agar (VWR Chemicals) for biochemical characterisation. *Salmonella* latex agglutination test (Oxoid) was used to confirm the genus of suspected colonies.

Listeria monocytogenes

Samples were analysed for the presence of *L monocytogenes* according to ISO 11290-1:2017,[26] with slight modifications. Briefly, for primary enrichment, 25 g of each samples was aseptically collected and transferred to a sterile blender bag, homogenised with 225 ml of half-concentrated Fraser broth (Oxoid) and incubated at 30°C±1°C for 24 hours. After primary enrichment, 0.1 ml of the cultures was transferred to 10 ml of Fraser broth (Oxoid) and incubated at 37°C±1°C for 48 hours for secondary enrichment. From both enrichment steps, 0.1 ml was spread onto agar Listeria Ottaviani and Agosti medium (Biolife Italiana, Milan). Plates were incubated for 48 hours at 37°C±1°C. Suspected *L monocytogenes* colonies, appearing with a green-blue colour surrounded by an opaque halo, were identified using the micromethod mono confirm test (Biolife Italiana).[27]

Campylobacter species

Samples were analysed according to ISO 10272-1:2017,[28] with slight modifications. Briefly, 25 g of each sample was aseptically collected and transferred to a sterile blender bag for primary enrichment with 225 ml of Bolton broth base (Oxoid). After homogenisation, samples were transferred to a sterile bottle and incubated under a microaerophilic atmosphere (Oxoid CampyGen 2.5 L Sachet, Oxoid) at 37°C for 4–6 hours and then at 41.5°C for 44 hours. After enrichment, 0.1 ml was spread onto blood free Campylobacter selectivity agar base (mCCDA, Oxoid) at 41.5°C for 44±4 hours under a microaerophilic atmosphere. Putative *Campylobacter* species colonies, appearing as flat/slightly raised, grey and wet/dry/hue spreading colonies, were analysed under phase-contrast microscope (100 x, Olympus) and M46 Microgen *Campylobacter* latex agglutination test (Microgen), which is able to detect the following species: *Campylobacter jejuni*, *C jejuni* subspecies *doylei*, *C coli*, *C upsaliensis*, *C laridis* and *C fetus*.

Results

Total aerobic bacteria count (TBC) ranged from a mean value of 4.22 x 10^4 colony-forming units (cfu)/g in sample 9 to 3.77 x 10^6 cfu/g in sample 16 (figure 1).

The total coliforms in the tested samples ranged from a mean value of 1.72 x 10^3 in sample 8 to 7.2 x 10^4 in sample 7 (figure 2). Presumptive *E coli* O157:H7 was isolated from 61 per cent of the total samples. However, after confirmation tests, 23 per cent of the samples were found to be contaminated by *E coli* O157:H7. For brand A, 22 per cent of the samples were confirmed to be contaminated by *E coli* O157:H7 (table 2). Of the samples from brand B, 16 per cent were found to

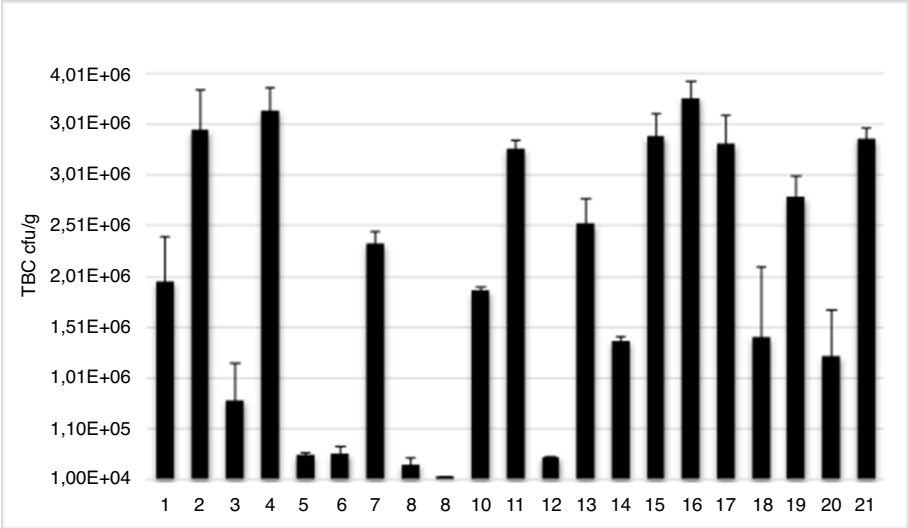

Figure 1 Total bacterial count (TBC) (mean values, cfu/g) in tested biologically appropriate raw food samples. Bars are sd. cfu, colony-forming units.

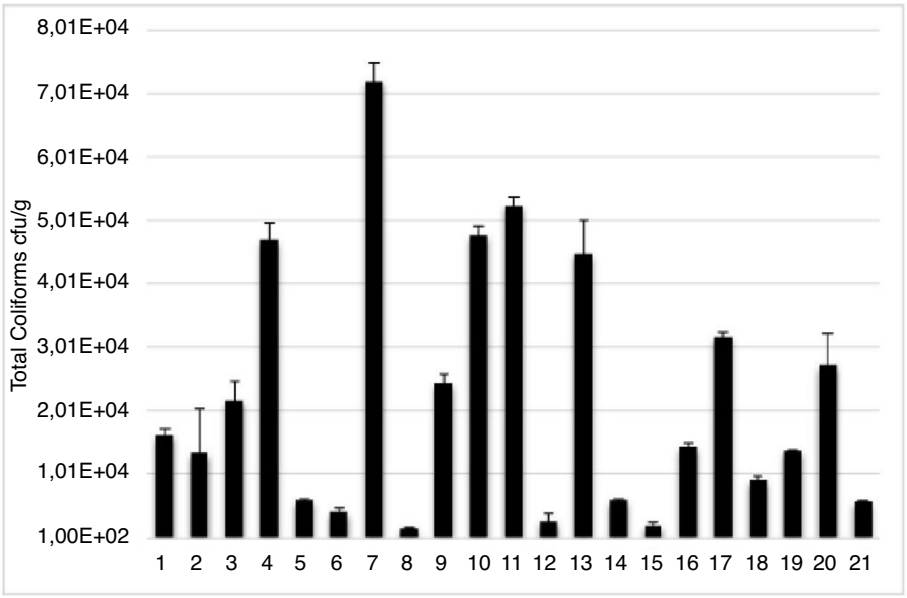

Figure 2 Coliforms (mean values, cfu/g) in tested biologically appropriate raw food samples. Bars are sd. cfu, colony-forming units.

be contaminated by *E coli* O157:H7. Finally, brand C showed *E coli* O157:H7 in 33 per cent of samples.

Salmonella species were isolated from 71 per cent of the samples: 56 per cent of the samples from brand A, and 83 per cent both from brand B and brand C (table 2).

L monocytogenes were isolated from 90 per cent of the tested samples: 88 per cent of the samples from brand A, 100 per cent from brand B and 83 per cent from brand C (table 2).

Finally, *Campylobacter* species were isolated from 29 per cent of the samples (22 per cent from brand A, 33 per cent both from brand B and brand C; table 2) despite their frozen status, which is known to limit the viability and cultivability of *Campylobacter* species.[29]

Discussion

The TBC results of the present study are in agreement with van Bree *et al*,[18] who analysed the presence of zoonotic bacteria and parasites in BARF diets for cats and dogs in the Netherlands, revealing TBC ranging from 7.9×10^2 to 5.0×10^6 cfu/g. In the present study the overall microbiological quality of the tested commercial products is acceptable according to the hygienic criteria applicable to both minced and mechanically separated meat intended for human consumption (Regulation EC No 2073/2005). Indeed, none of the samples showed TBC higher than 5×10^6 cfu/g, which is the limit to consider a sample unacceptable. However, 14 out of 21 samples showed TBC higher than 5×10^5 cfu/g, which is the limit to consider a sample marginally acceptable.

As for coliforms, the results of the present study are in agreement with Weese *et al*,[30] who in analysing 25 commercial raw diets for dogs and cats found coliform contamination ranging from 3.5×10^3 to 9.4×10^6 cfu/g. Also other previous studies have highlighted high frequencies and levels of coliform contamination in raw meat-based diets.[31 32] Coliforms provide an indication of the general microbiological condition of food, and among them *E coli* is an indicator of faecal contamination, informing on the hygienic quality of the sample.

With regard to detection of *E coli* O157:H7, the results of the present study are in good agreement with the study of van Bree *et al*,[18] where *E coli* O157:H7 was found in 23 per cent of the tested samples and almost 80 per cent of the samples were contaminated by ESBL producer *E coli*. Similar results were also obtained by Nilsson,[32] who isolated from all the tested samples *E coli* positive for the bla_{CMY-2} family of the ampC beta-lactamase genes, which are known to confer broad-spectrum resistance to beta-lactamases antimicrobials.[33] Some studies have reported an increase in the antimicrobial resistance patterns of *E coli* O157:H7.[34–36] Therefore, the number of positive *E coli* O157:H7 samples found in the present study confirms that, together with the risk associated with the presence of one of the most important foodborne pathogens among Shiga toxin-producing *E coli*, the use of BARF products could also spread antibiotic resistance genes among pets and owners.[20 32 37] *E coli* O157: H7 has a very low infective

Table 2 Samples contaminated by presumptive *Escherichia coli* O157, *E coli* O157:H7, *Listeria monocytogenes*, *Salmonella* species and *Campylobacter* species among tested BARF products

Brand	Presumptive *E coli* O157, n (%)	*E coli O157:H7*, n (%)	*L monocytogenes*, n (%)	*Salmonella* species, n (%)	*Campylobacter* species, n (%)
A	5 (56)	2 (22)	8 (88)	5 (56)	2 (22)
B	2 (33)	1 (16)	6 (100)	5 (83)	2 (33)
C	6 (100)	2 (33)	5 (83)	5 (83)	2 (33)

BARF, biologically appropriate raw food.

dose, less than 50 cells/g in people[38]; thus, simply handling contaminated pet foods could expose owners to a relevant risk of infection. Cross-contamination is quite a likely event during food preparation,[39] even if it is possible that owners do not simultaneously prepare food for themselves and their pets and that they wash their hands and clean the kitchen table before preparing food. Furthermore, infected pets can be asymptomatic carriers and could directly infect their owners.[40]

The results obtained for *Salmonella* species were in agreement with Joffe and Schlesinger,[41] who found 80 per cent of raw pet diets were contaminated by *Salmonella* species. However, the results of the present study are much higher than those reported by van Bree *et al*[18] and by Fredriksson-Ahomaa *et al*,[10] where only 20 per cent and 2 per cent of the samples, respectively, tested positive for *Salmonella* species. This discrepancy could be due to the lower prevalence of *Salmonella* species in Finnish and Dutch farm animals compared with Italy and Germany, where the meat sampled for this study came from.[42–44]

Previous studies suggest that *Salmonella* species can persist at room temperature in contaminated food bowls, and that cleaning and disinfection of these bowls may not eliminate *Salmonella*.[45] Furthermore, as for *E coli*, pets that consume contaminated raw food diets can be colonised with *Salmonella* species without exhibiting clinical signs, making them a possible source of contamination.[15 46] It also has to be noted that animals fed with dry foods could carry *Salmonella* in their faeces, although transmission from dogs to people has rarely been reported.[47] However, a systematic review of case–control studies has shown that direct contact with pets plays a major role in human salmonellosis, and direct transmission has been frequently reported.[48]

The results of this study highlight that, also with *Salmonella* species, BARF products sold in Italy could present a potential threat to owners' health if products are not hygienically handled. The results were in good agreement with other studies[18] with regard to the presence of *L monocytogenes* in BARF diet samples. It is not surprising that *L monocytogenes* is the most widespread pathogen in this type of food, as the conditions of production, storage and use of these products allow the development and uncontrolled proliferation of this microorganism. *L monocytogenes* is in fact a psychotropic and ubiquitous microorganism.[49] The ability to survive and grow under refrigerated temperatures means that products that do not undergo heat treatment, such as BARF diet products, can be a source of listeriosis. In addition, once raw pet food items are purchased, they may be exposed to increased temperatures during transport, and after arrival at home encourage the potential growth of pathogens.

Listeriosis is a serious disease in people, and with the possibility of domestic animals being asymptomatic, infected pets could be a direct source of infection.

Finally, the results of this study revealed the presence of *Campylobacter* species in the samples, which was higher than expected considering the samples' frozen nature. However, several studies showed that *Campylobacter* species may be more robust than previously thought and can survive freezing and thawing.[50–52] There remains uncertainty about the minimum infectious doses for *Campylobacter* species,[53] but some estimates are as low as 500 cells/g, and therefore simply handling contaminated pet foods could expose owners to risk of infection.

Taken together, the results of the present study show that the frozen BARF products that were analysed had high levels of microbial contamination, beyond the microbiological limits set by the EU regulation for products that are intended for human consumption. However, the limited sample size considered in the present study might not represent the overall situation in all raw food products sold in Italy. Dedicated legislation is not available yet for BARF pet foods, but their microbiological quality should fall, at least, within the specification for human products. Given that raw feeding is currently well established and that BARF diet meals may be prepared at home, probably in the kitchen, using hands and tools that could be shared, specific microbiological criteria should be set to limit the risk to pet owners. It would even be recommendable to have some EU regulations for such products, including specific microbial limits and labelling that contains guidelines for consumers. These guidelines should include suggestions to consult veterinarians for advice on the most appropriate diet for their pets; however, in this context, pet owners should also be made aware of the potential risks of BARF feeding strategy both to themselves and to their pets. Furthermore, no information on safe handling of these raw meat products was available on the packaging of the tested BARF products. This is a significant omission that can only be partially addressed by guidelines on safer handling of BARF products at home as recommended by veterinarians and nutritionists.

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